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Malignancy Rate of Thyroid Nodules Defined as Follicular Lesion of Undetermined Significance and Atypia of Undetermined Significance in Thyroid Cytopathology and Its Relation with Ultrasonographic Features

Neslihan Çuhaci · Dilek Arpaci · Rıfki Üçler · Aylin Kilic Yazgan · Gülten Kıyak · Samet Yalçin · Pamir Eren Ersoy · Gülnür Güler · Reyhan Ersoy · Bekir Çakir

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Abstract Fine-needle aspiration biopsy (FNAB) has been widely accepted as the most accurate, safe, and cost-effective method for evaluation of thyroid nodules. The most challenging category in FNAB is atypia of undetermined significance (AUS) and follicular lesion of undetermined significance (FLUS). The Bethesda system (BS) recommends repeat FNAB in that category due to its low risk of malignancy. In our study, we aimed to investigate the malignancy rate of thyroid nodules of AUS and FLUS and whether there were different malignancy rates among the different patterns in this category, and to evaluate the presence of biochemical, clinical, and echographic features possibly predictive of malignancy related to AUS and FLUS. Data of 268 patients operated for AUS and FLUS cytology were screened retrospectively. Ultrasonographic features and thyroid function tests, thyroid antibodies, scintigraphy, and histopathological results were evaluated. Of the 268 patients' results, 276 nodules are evaluated. Malignancy rates were 24.3 % in the AUS group, 19.8 % in the FLUS group, and 22.8 % in both groups. In the evaluation of all nodules, the predictive features of malignancy are hypoechogenicity and peripheral vascularization of the nodule. We determined that the malignancy rates in these nodules are higher than that in the literature rate. This high ratio may be due to the fact that we studied only patients who underwent surgery. The ultrasonographic features alone may be insufficient to predict the malignancy; therefore, all the clinical and ultrasonographic features must be considered in the evaluation of the thyroid nodules. In addition, we think that the recommended management of repeat FNAB in these groups must be reconsidered with the clinical and ultrasonographic features.

Keywords Atypia of undetermined significance (AUS) · Follicular lesion of undetermined significance (FLUS) · Thyroid nodule · Malignancy · Ultrasonography (USG)

N. Çuhaci (✉) · D. Arpaci · R. Üçler
Department of Endocrinology and Metabolism, Atatürk Education and Research Hospital, Ankara, Turkey
e-mail: neslihan_cuhaci@yahoo.com

A. K. Yazgan
Department of Pathology, Atatürk Education and Research Hospital, Ankara, Turkey

G. Kıyak · P. E. Ersoy
Department of General Surgery, Atatürk Education and Research Hospital, Ankara, Turkey

S. Yalçin
Faculty of Medicine, Department of General Surgery, Yildirim Beyazit University, Ankara, Turkey

G. Güler
Faculty of Medicine, Department of Pathology, Yildirim Beyazit University, Ankara, Turkey

R. Ersoy · B. Çakir
Faculty of Medicine, Department of Endocrinology and Metabolism, Yildirim Beyazit University, Ankara, Turkey

Introduction

Nodular thyroid disease is a common endocrine disorder that is particularly prevalent in iodine-deficient regions [1, 2]. Although thyroid nodules are often benign, the malignancy rate is 5–17 % and is increasing steadily [3–7]. Malignancy

risks in patients with thyroid nodules include radiation history to the head and neck, family history of medullary thyroid cancer, multiple endocrine neoplasia type II and papillary thyroid cancer, age <14 and >70 years old, male sex, growing and hard nodules, cervical lymphadenopathy, fixed nodules, as well as dysphonia, dysphagia, and dyspnea [8].

High-resolution (10 MHz) ultrasonography (USG) is a useful method for evaluating thyroid nodule morphology, lymph nodules, nodule follow-up, and guidance for fine-needle aspiration biopsy (FNAB) [9]. The American Radiology Association has defined the ultrasonographic features of nodules associated with malignancy as hypoechogenicity of the nodule, absence of a peripheral halo, presence of microcalcification, irregular border, anteroposterior diameter/transverse diameter >1, and increased central vascularity [10]. The sensitivity (Sn), specificity (Sp), and accuracy of these features vary among studies [3]. Therefore, the use of USG alone is not sufficiently sensitive or specific to identify malignant thyroid nodules. However, the nodule features mentioned above increase the malignancy rate [3].

Thyroid FNAB is the primary method for identifying malignancy in patients with thyroid nodules [11]. In iodine-sufficient regions, Sn and Sp of this method are both >90 % [9]. In 2007, the National Cancer Institute (NCI) organized the NCI Thyroid Fine Needle Aspiration State-of-the-Science Conference and discussed various aspects of thyroid FNAB [12]. Consequently, the Bethesda system (BS) for reporting thyroid cytopathology was described at this conference. According to this terminology, FNAB results are divided into six categories: (1) nondiagnostic, (2) benign, (3) atypia/follicular lesion of undetermined significance (AUS/FLUS), (4) follicular neoplasm/suspicious of follicular neoplasm, (5) suspicious of malignancy, and (6) malignant [13, 14]. By definition, AUS/FLUS involves a heterogeneous category that includes structural, cellular, and nuclear atypia of the samples [11]. The BS recommends that use of this category be limited and should not exceed 7 % of the diagnoses within a given laboratory [13, 15]. The malignancy rate in this category has been estimated to be 5–15 % [14]. The aim of this category was to group lesions that were not necessarily suspected of representing a follicular carcinoma (high cellularity/scant colloid, microfollicular/trabecular arrangements) but did not meet the criteria for the benign or malignant categories [16]. This category includes lesions that are not easily classified as benign, suspicious, or malignant [16]. This system makes it possible to repeat the biopsy for patients who have follicular atypia of a thyroid nodule. If the same diagnosis is determined (20–25 % of cases) then surgery is usually recommended [14].

There are no highly sensitive and specific diagnostic procedures available to evaluate indeterminate thyroid nodules [17]. In these cases, the radiologic and cytologic features as well as the expression of cell markers are proposed to serve as diagnostic adjunctive tools in the evaluation of such nodules, but they have limited relevance [18]. The NCI conference session on ancillary techniques provided no recommendations for indeterminate lesions [19]. Potential problems included the limited data available for immunohistochemistry (IHC) in large cohort studies and the different methods used to obtain cytological specimens for immunocytochemistry (ICC) [20, 21]. The conclusions from the conference were that IHC/ICC should be considered for some suspected malignancies and for some specific diagnoses (thyroid nodule vs. parathyroid nodule), but there was insufficient evidence to determine a specific recommendation for indeterminate/suspicious FNAB [20, 21]. However, several studies have shown that IHC markers may improve the accuracy of cytologic diagnosis. Fadda et al. showed that HBME-1 and galectin-3 are positive in 83.3 % of the cases resulting in malignancy and are negative in 87.5 % of cases resulting in benign histopathology [20]. Among the cases diagnosed as follicular neoplasia (FN) and AUS, a completely positive immunocytochemical panel was found in 76.9 % of cases resulting in malignancy, and a complete negative panel was found in 96.8 % of cases resulting in benign histology [20]. They found that HBME-1 had a better diagnostic accuracy than galectin-3 and suggested applying these markers in the cytological diagnosis of FN/AUS to determine the true preoperative diagnosis of thyroid nodules [20]. Cochand-Priollet et al. [21] found that 4 % of ICC results are false positives, but none false negative, for indeterminate thyroid lesions. In indeterminate thyroid lesions, they found that the Sn, Sp, negative predictive value (NPV), and positive predictive value (PPV) for malignant–benign lesions were 100, 85.2, 100, and 86.2 %, respectively. They suggested that ICC of thyroid FNAB using cytokeratin 19 and HBME-1 antibodies can reduce the false-positive and false-negative results of single morphological analyses, consequently improving the diagnostic accuracy and reducing the need for surgical controls [21]. Nikiforov et al. [6] studied 1,056 consecutive thyroid FNA samples with indeterminate cytology (to determine BRAF, RAS, RET/PTC, and PAX8/PPARγ) and found that detecting any mutations in the atypia/FLUS group was consistent with 88 % malignancy, whereas the risk of malignancy was 14 % when based only on cytology. Therefore, they recommended total thyroidectomy in mutation-positive cases and lobectomy in mutation-negative cases.

The AUS/FLUS category includes heterogeneous patterns of atypia. Some authors suggest that the different patterns are associated with a higher risk of malignancy [22–25]. In this study, we evaluated the malignancy rates in the AUS/FLUS category and determined whether there were different malignancy rates among the different patterns in this category. We also evaluated the presence of biochemical, clinical, and

echographic features that are potentially predictive of malignancy related to AUS and FLUS.

Materials and Methods

Patients

Patients who visited our endocrinology clinics between January 2010 and March 2012, who were found to have at least one thyroid nodule by USG, underwent USG-guided FNAB because of solitary nodules or multinodular goiter according to current guidelines [8], and whose cytology was identified as AUS/FLUS according to the BS were evaluated retrospectively. Patients who had suspected ultrasonographic features or clinical features such as large nodule size or growth, mass effect, and a family history of thyroid cancer underwent surgery. Patients were divided into groups according to thyroid function test results: euthyroid, hypothyroid, hyperthyroid, subclinical hypothyroid, and subclinical hyperthyroid. Patients with overt hyperthyroidism were divided into two groups according to their scintigraphic results: toxic nodular or multinodular goiter, and toxic diffuse nodular or multinodular goiter.

Exclusion Criteria

Patients <15 years and those who had a history of thyroid operation, percutaneous intervention, or radiotherapy of the head and neck were excluded from the study. Patients who had a contraindication for surgery due to comorbid diseases (cardiovascular or respiratory system diseases) or who refused surgery were also excluded.

Laboratory

The levels of sensitive thyroid-stimulating hormone (sTSH), free triiodothyronine (fT3), free tetraiodothyronine (fT4), thyroid autoantibodies (thyroid peroxidase antibody [anti-TPO] and thyroglobulin antibody [anti-Tg]), and thyroglobulin were measured in all patients using chemiluminescence methods (Immulite 2000, Diagnostic Products Corp., Los Angeles, CA, USA and UniCel DXI 800, Beckman Coulter, Brea, CA). The normal ranges for sTSH, fT3, fT4, anti-Tg, and anti-TPO were 0.4–4 μIU/mL, 1.57–4.71 pg/mL, 0.61–1.12 ng/dl, <30 U/mL, <10 U/mL, and 0–55 ng/mL, respectively. Suppressed TSH levels with normal free thyroid hormone levels were described as "subclinical hyperthyroidism," and suppressed TSH levels with high free hormone levels were described as "overt hyperthyroidism." In contrast, elevated TSH with normal free thyroid hormone levels was categorized as "subclinical hypothyroidism," and elevated TSH with reduced hormone levels was described as "over thypothyroidism."

Conventional USG and Scintigraphy

Esaote color Doppler USG (Model 796FDII; MAG Technology Co. Ltd., Yung-Ho City, Taipei, Taiwan) and standard USG with a superficial probe (Model LA523 13–4, 5.5–12.5 MHz) were used. The patient was in a supine position with their neck hyperextended, and the skin was coated with acoustic material. Localization, diameter (in milliliter), and volume of the nodule; components; echogenicity; border regularity; calcification; and the presence of a peripheral halo were evaluated by conventional USG. Parenchyma of the thyroid gland was evaluated as homogenous or heterogeneous. Echogenicity was evaluated as hypoechoic, isoechoic, iso-hypoechoic, or hyperechoic. Nodule components were described as solid (no cystic component), mixed (containing cystic part), or pure cystic (almost cystic or very little solid part). Calcification of the nodule was defined as microcalcification, macrocalcification, micro–macrocalcification, or no calcification.

^{99m}Tc pertechnetate was used for the scintigraphic evaluation of the thyroid gland. The patients were administered 5 mCi of ^{99m}Tc pertechnetate intravenously 20–40 min after radioiodine administration. Thyroid scintigraphy was carried out using a gamma camera with a pinhole collimator. Of the 276 patients, thyroid scintigraphy was applied to 71 (80.6 %) in the FLUS group and 57 (12.1 %) in the AUS group. According to the scintigraphic results, nodules were divided into hypoactive, hyperactive, and normoactive groups.

USG-Guided FNAB

FNAB was performed with USG using a General Logic Pro 200 system (model 2270968; GE Healthcare, Seoul, Korea and Seongnam SI, Gyeonggi-do, Korea) and a 5.5–7.5-MHz superficial probe. Written consent was obtained from all patients prior to FNAB. No anesthetics were administered before the procedure. USG-guided FNAB was performed in all patients by an experienced endocrinologist using a 23-gauge needle and 20-mL syringe.

Cytological and Histopathological Examination

Materials obtained by USG-guided FNAB were air dried, stained with May–Grünwald–Giemsa, and evaluated according to the BS classification [12]. The cytopathological diagnosis was made on conventional smears. Cell blocking was not performed. Accordingly, the cytology results were as follows: (1) nondiagnostic, (2) benign, (3) AUS/FLUS, (4) follicular neoplasm/suspicious for follicular neoplasm, (5) suspicious for malignancy, and (6) malignant [13, 14].

Although AUS/FLUS was categorized using the same classification as the BS, conventional smears of AUS/FLUS cases were subclassified into two patterns based on cytomorphology.

AUS cases were defined as having an atypical cell pattern (could not exclude papillary thyroid carcinoma, such as the presence of occasional nuclear grooves, an abnormal chromatin pattern, or nuclear overlapping and crowding) (Fig. 1), and FLUS cases were defined as a microfollicular pattern with low cellularity and no or minimal colloid (could not exclude follicular and Hurthle cell neoplasm) (Fig. 2).

The histopathological evaluation was made according to the 2004 World Health Organization criteria [26]. The differential diagnoses for the final histopathological diagnosis were made using specimens stained immunohistochemically for HBME-1, galectin-3, Ki-67, and/or CK19.

Statistical Analysis

Shapiro–Wilks tests were used for the constant variables. Descriptive statistics for the constant variables are expressed as mean±standard deviation or median (range), and categorical variables are noted as numbers and percent.

Significant differences between the means of different groups were evaluated by Student's *t* tests, and significant differences in median values were evaluated by Mann–Whitney *U* tests. Categorical variables were evaluated by Pearson's chi-square or Fisher's tests. The odds ratio and 95 % confidence intervals of all factors that could affect malignancy rates were evaluated.

Following the univariate statistical analysis, risk factors with $p<0.025$ were subjected to a multivariate model. According to the gradual logistic regression analysis, the most predictive risk factors for malignancy were determined. p values≤ 0.05 were considered statistically significant.

Results

In total, 276 nodules from 268 patients were evaluated; 219 (81.7 %) patients were female and 49 (18.3 %) were male. The mean age (range) of the patients was 47.85±11.64 (15–74) years. FNAB samples were divided into the AUS and FLUS groups according to the BS. The mean age of the patients was 48.38±11.41 (15–74) years in the AUS group and 46.76±12.1 (17–74) years in the FLUS group. In the AUS group, 150 (83.3 %) patients were female and 30 (16.7 %) were male, and in the FLUS group, 69 patients (78.4 %) were female and 19 (21.6 %) were male. No significant differences were observed between the two groups in terms of age or gender.

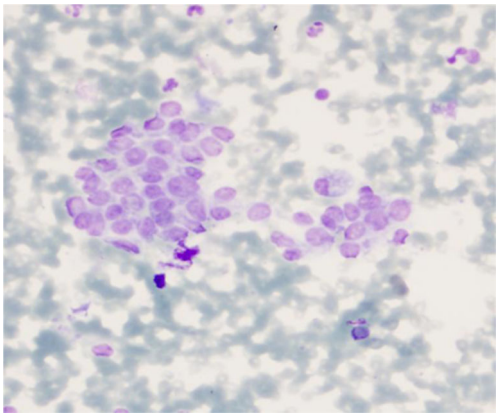

Fig. 1 May–Grunwald–Giemsa (MGG) staining. Atypical cells with nuclear membrane irregularity and with nuclear larging (×40 objective)

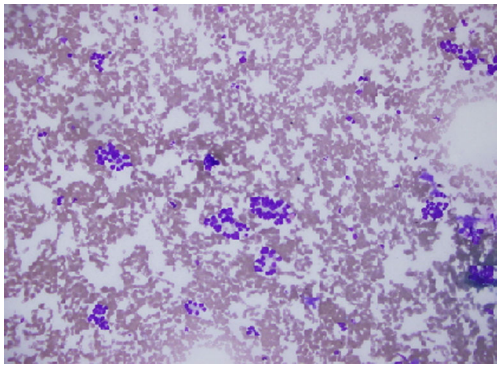

Fig. 2 May–Grunwald–Giemsa (MGG) staining. Hypocellular and microfollicular pattern of the cells (×40 objective)

According to the thyroid function test and USG results, euthyroid multinodular goiter was the most common finding. In the AUS group, 185 nodules from 180 patients, and in the FLUS group, 91 nodules from 88 patients were evaluated retrospectively. According to the thyroid scintigraphy results, more hypoactive nodules tended to be seen in the FLUS group than those in the AUS group, but the difference was not significant. The sonographic features of the nodules were not different between the two groups. In the AUS group, the mean transverse diameter of nodules was 21.73±13.5 mm in the benign group and 19.09±9.79 mm in the malignant group; in the FLUS group, the values were 21.98±11.02 and 27.65±18.4 mm, respectively. These differences were not significant.

Histopathologic Results

The histopathologic findings are listed in Table 1.

Table 1 Comparison of histopathologic results between groups

	Histopathologic results		*p*
	Benign *n* (%)	Malignant *n* (%)	
AUS	140 (75.7)	45 (24.3)	0.398
FLUS	73 (80.2)	18 (19.8)	
AUS + FLUS	213 (77.2)	63 (22.8)	

AUS atypia of undetermined significance, *FLUS* follicular lesion of undetermined significance

The most common malignancy was papillary thyroid carcinoma (PTC) (Fig. 3), and the most common PTC subtype was follicular variant of PTC in all groups. No significant differences in vascular, capsular, extracapsular, lymph node metastasis, or multicentricity were observed in the malignant histopathologic groups among AUS, FLUS, or AUS + FLUS. Mean tumor size was 1.64±1.39 (0.1–6) cm. No significant difference in tumor size was observed between AUS and FLUS. No significant difference was observed between single and repeat FNAB after comparing the benign (Fig. 4) and malignant histopathologic results according to repeated FNAB.

Mean age was significantly lower in the malignant group than that in the benign group in all histopathologic groups (p= 0.01). No significant differences were observed for sex, thyroid function tests, or the scintigraphic results.

Hypoechoic nodule rates and peripheral vascularization rates were significantly higher in the malignant group than those in the benign group according to the ultrasonographic features (p values=0.02 and 0.011, respectively). Isoechoic nodule rates were significantly higher in the FLUS group (77.8 %) than those in the AUS group (48.9 %) (p=0.036). The ultrasonographic features of the malignant and benign groups are presented in Table 2.

Predictive factors for distinguishing between malignant and benign histopathologic results were evaluated by multivariate logistic regression analysis. Age, sex, thyroid function tests, thyroid antibody positivity, nodule size, and ultrasonographic features of the nodules were evaluated. The results are given in Table 3.

According to the multivariate logistic regression analysis, the only predictive features of malignancy were hypoechogenicity in the AUS group (PR 4.714, 95 % CI 1.732–12.835, p=0.004), and peripheral vascularization in the FLUS group (odds ratio 3.460, 95 % CI 1.0007–11.886, p=0.049).

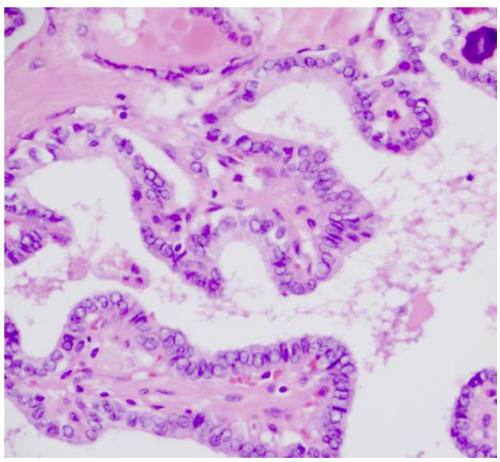

Fig. 3 Classical variant of papillary thyroid carcinoma (with hematoxylin–eosin staining) (×40 objective)

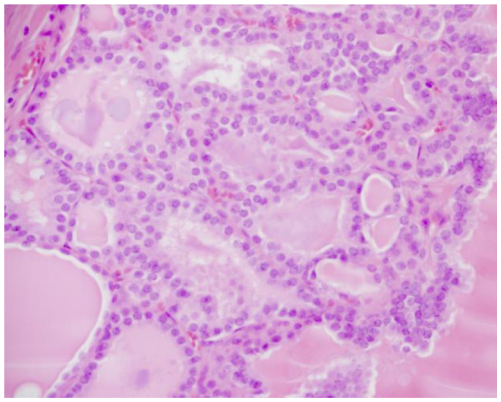

Fig. 4 Colloidal nodule with hyperplastic changes (with hematoxylin–eosin staining) (×40 objective)

Discussion

We investigated the malignancy rate of AUS/FLUS thyroid nodules and whether there were different malignancy rates among the different patterns in this category. We also evaluated the presence of biochemical, clinical, and echographic features that were potentially predictive of malignancy related to AUS and FLUS. The BS recommends that use of this category be limited and should not exceed 7 % of the diagnoses within a given laboratory [13, 15]. In another study which is carried out in our center, revealed that our institution's overall AUS/FLUS diagnostic rate was 4.8 % [27]. We found malignancy rates of 24.3 % in AUS, 19.8 % in FLUS, and 22.8 % in the AUS + FLUS groups. The malignancy rate was not different between the groups. The most common malignancy was PTC in the AUS group, whereas it was PTC and well-differentiated thyroid neoplasm in the FLUS group. The most common PTC subtype in both groups was the follicular variant.

The malignancy rate for AUS/FLUS is thought to be 5–15 % [11], but the true rate is uncertain because not all nodules are subjected to histopathological evaluation. In previous studies that included a cytologic follow-up, the malignancy rate was low, but in a large series, it was 27.5 % [28]. The malignancy rate was 6–48 % among operated cases [11, 29]. In one study, the malignancy rate was 25 % in indeterminate cytology [30], yet in another study, it was 21.8 % [2]. Because fewer patients underwent surgery, the actual malignancy rate in the FLUS group is conjectural, although it has been reported to be 0–35 % [18, 31]. Nayar et al. reported a malignancy rate of 6 % [32], Yassa et al. reported 24 % [33], and Yang et al. reported 19 % [34] in a FLUS group. In a AUS group, the malignancy rate was 25 % in patients who underwent operations. Nevertheless, the actual incidence is 5–10 % in nonoperated cases [31]. The malignancy rates of AUS/FLUS reported in the literature are listed in Table 4.

In our study, the malignancy rate of AUS/FLUS was 22.8 %, which was similar to some values in the literature [2, 16, 30, 38], although this ratio was higher than that

Table 2 Ultrasonographic features of the malignant and benign histopathologic results

USG features		AUS		*p*	FLUS		*p*
		Benign *n* (%)	Malignant *n* (%)		Benign *n* (%)	Malignant *n* (%)	
Component	Cystic	3 (2.1)	–	1.0	4 (5.5)	–	0.581
	Solid	54 (38.6)	55* (55.6)	**0.045**	26 (35.6)	6 (33.3)	0.856
	Mix	83 (59.3)	20 (44.4)	0.081	43 (58.9)	12 (66.7)	0.546
Echogenicity	Isoechoic	84 (79.2)	22 (20.8)	0.19	41 (74.5)	14 (25.5)	0.093
	Hypoechoic	8 (44.4)	10** (55.6)	**0.003**	8 (88.9)	1 (11.1)	0.682
	Iso-hypoechoic	46 (78)	13 (22)	0.682	22 (88)	3 (12)	0.546
	Hyperechoic	2 (100)	–	1.0	2 (100)	–	1.0
Border regularity	Regular Irregular	66 (47.1) 74 (52.9)	18 (40) 27 (60)	0.402	36 (49.3) 27 (50.7)	8 (44.4) 10 (55.6)	0.711
Calcification	Absent Present	98 (70.9) 42 (30)	31 (68.7) 14 (30.3)	1.0	56 (76.7) 17 (23.3)	9 (50) 9**** (50.0)	**0.03**
Halo	Present Absent	60 (42.9) 80 (57.1)	21 (46.7) 24 (53.3)	0.654	29 (39.7) 44 (60.3)	8 (44.4) 10 (55.6)	0.715
Peripheral vascularization	Present Absent	24 (17.1) 116 (82.9)	14 (31.1) 31*** (68.9)	**0.044**	15 (20.5) 58 (79.5)	7 (38.9) 11 (61.1)	0.128
Peripheral macrocalcification	Present Absent	1 (0.7) 139 (99.3)	– 45 (100)	0.57	– 73 (100)	– 18 (100)	>0.05
Nodule localization	Right lobe Left lobe	84 (60) 51 (36.4)	25 (55.6) 20 (44.4)	0.178	31 (42.59) 34 (46.6)	10 (55.6) 8 (44.4)	0.281
	Isthmus	5 (3.6)	–		8 (11)	–	

Values in bold are to highlight the statistical significance

*p=0.045; **p=0.003; ***p=0.044; ****p=0.03

determined by the BS. Our malignancy rate for FLUS (19.8 %) was higher than that reported by Nayar et al. [32], lower than that of Yassa et al. [33], and similar to that of Yang et al. [34]. Our rate was higher than the BS probably because we studied patients who underwent surgery. Another factor contributing to the high ratio may be the heterogeneity of AUS/FLUS. Additionally, some studies predate the BS and then adopted the BS [18]. Furthermore, specimen preparation methods could contribute to such differences [28], and different patterns of AUS/FLUS may have different malignancy risks [11]. Some authors have suggested that different patterns are associated with a higher risk of malignancy [22–25].

Table 3 Predictive factors for differentiating between malignant and benign histopathologic results

Variables	Odds ratio	95 % confidence interval		*p*
		Lower limit	Upper limit	
Age	0.963	0.939	0.989	**0.005**
Anti-Tg positivity	0.466	0.220	0.984	**0.045**
Hypoechogenicity	2.461	1.034	5.859	**0.042**
Peripheral vascularization	2.198	1.133	4.266	**0.020**

Values in bold are to highlight the statistical significance

Önder et al. evaluated the frequency and malignancy outcomes of indeterminate categories and classified AUS/FLUS into four subcategories: PTC pattern, microfollicular pattern, Hurthle cell pattern, and atypical cell pattern [39]. They found that the malignancy rates for the PTC, atypical cell, and microfollicular pattern were 28, 22.2, and 6.9 %, respectively. The Hurthle cell category did not show any malignant lesions [39]. Renshaw et al. [23] reported a malignancy rate of 38 % in AUS/FLUS with PTC features, whereas Luu et al. [22] and Olson et al. [24] reported malignancy rates of 45.8 and 48 %, respectively, for this category. Choi et al. [40] subclassified the AUS/FLUS category into two subcategories according to previous studies [24, 31, 35, 41]: nuclear atypia (AUS group) and low cellularity with a predominant microfollicular pattern

Table 4 Malignancy rates of thyroid nodules defined as AUS/FLUS by FNAB

Study	*n*	Malignancy rate (%)
Theoharis et al. [35]	89	48
Layfield et al. [36]	127	28
Rabaglia et al. [37]	91	13
Faquin and Baloch [38]	273	19
Broome et al. [16]	82	20

and no or minimal colloid (FLUS group). They showed that the AUS group had a significantly higher risk of malignancy (65 vs. 14.3 %) as well as malignant USG findings (76.5 vs. 52.7 %) compared with those in the FLUS group. The total malignancy rate was 32.4 % [40]. They recommended that clinical follow-up may be adequate for managing FLUS nodules [40]. They also suggested that the guidelines for managing the AUS/FLUS category clinically may be further reevaluated because of the different malignancy risks, management recommendations, and malignant USG findings between the two groups [40]. Considering all of these factors, we conclude that the definition of this category in the BS does not reflect agreement between pathologies.

We compared the findings of repeat FNAB and found no statistically significant difference between the benign and malignant histopathological groups. In a previous study, patients with a single AUS diagnosis who went directly to surgery showed similar malignancy rates to those who had two consecutive AUS diagnoses [28]. Another study reported that the malignancy rate was 19 % in AUS/FLUS and 27 % in the repeat FNAB group [38].

We found that the mean age of the patients was younger in the malignant group than that of the benign group in the total AUS + FLUS and AUS groups, whereas no significant difference was observed in the FLUS group. No significant differences were observed between histopathologic groups and sex, thyroid function tests, Tg levels, or scintigraphic features of nodules in the AUS, FLUS, and AUS + FLUS groups. In a previous study, which was similar to our study, no differences between histopathology results and sex and thyroid function tests were observed [31]. Nevertheless, there is no agreement among studies for the relationship between age and malignancy rate. In one study, the malignancy rate was higher in patients <40 years old [42]. Similarly, in another study [43], patients with thyroid malignancy were younger than patients in the benign group. However, other studies have suggested the opposite, reporting higher rates of thyroid malignancy with increasing age [5, 44].

Studies have suggested that nodule size may assist in cancer risk assessment, although data are conflicting, and some studies have claimed that nodule size is not associated with malignancy rate [45–50]. Although similar to previous findings [18, 30, 31, 42, 51, 52], we did not observe significant differences between nodule diameter and malignancy rate. In a large series of nodules with indeterminate or suspicious cytology, a threshold effect was detected at a nodule diameter of approximately 2.5 cm, and it has been suggested that the malignancy risk increases by up to 39 % for every 1 cm increase in nodule size [53].

Gray-scale ultrasonographic patterns suggesting malignancy include solid hypoechoic appearance, presence of microcalcification, and being anteroposterior or with a transverse diameter of >1 [43]. Our results revealed that among the ultrasonographic features in the AUS/FLUS group, hypoechogenicity and peripheral vascularization were significantly higher in the malignant group. In the AUS group, hypoechogenicity, peripheral vascularization, and the solid component were significantly higher in the malignant group. Isoechoic nodule rates were higher in the FLUS group than in the AUS group, but the difference was not statistically significant. Rago et al. [2] reported that the malignancy rate in nodules with indeterminate cytology was 21.8 %, and that gray-scale USG alone was insufficient for distinguishing between malignant and benign lesions. In that study, the absence of a halo and the presence of microcalcification features were the most relevant to malignancy. The main objective of our study was to evaluate the biochemical, clinical, and echographic features one by one in AUS, FLUS, and in both groups related to malignancy.

The limitations of the present study are that it was retrospective and we selected patients who had undergone an operation. The predictive features of malignancy included hypoechogenicity and peripheral vascularization of the nodule in the total AUS + FLUS group, but only hypoechogenicity in AUS and peripheral vascularization in the FLUS group. These findings support that ultrasonographic features alone may be insufficient for predicting malignancy; therefore, all clinical and ultrasonographic features must be considered. Another restriction of our study was the lack of immunohistochemical markers. As mentioned above, markers may be promising for diagnosing thyroid nodules, particularly in cytologically indeterminate lesions.

The suggested recommendation is repeat FNAB for cases that have AUS/FLUS cytology, due to the low malignancy rates, except pure cystic cases [11]. This approach is considered safe and cost effective [11]. Clinical and radiological correlations are also important [11]. However, we revealed no difference in malignancy rate between single and repeat FNAB, and that the overall malignancy rate was higher than expected. Therefore, we suggest that surgery may be conceivable earlier instead of repeat FNAB especially with suspected ultrasonographic features.

References

1. Cakir B, Aydin C, Korukluoğlu B, Ozdemir D, Sisman IC, Tüzün D, Oguz A, Güler G, Güney G, Kuşdemir A, Sanisoglu SY, Ersoy R (2011) Diagnostic value of elastosonographically determined strain index in the differential diagnosis of benign and malignant thyroid nodules. Endocrine 39:89–98
2. Rago T, Scutari M, Santini F, Loiacono V, Piaggi P, Di Coscio G, Basolo F, Berti P, Pinchera A, Vitti P (2010) Real-time elastosonography: useful tool for refining the presurgical diagnosis in thyroid nodules with indeterminate or nondiagnostic cytology. J Clin Endocrinol Metab 95:5274–5280
3. Moon WJ, Baek JH, Jung SL, Kim DW, Kim EK et al (2011) Ultrasonography and the ultrasound-based management of thyroid nodules: consensus statement and recommendations. Korean J Radiol 12:1–14

4. McCoy KL, Jabbour N, Ogilvie JB, Ohori NP, Carty SE, Yim JH (2007) The incidence of cancer and rate of false-negative cytology in thyroid nodules greater than or equal to 4 cm in size. Surgery Dec 142:837–844
5. Carrillo JF, Frias-Mendivil M, Ochoa-Carrillo FJ, Ibarra M (2000) Accuracy of fine-needle aspiration biopsy of the thyroid combined with an evaluation of clinical and radiologic factors. Otolaryngol Head Neck Surg 122:917–921
6. Nikiforov YE, Ohori NP, Hodak SP, Carty SE, LeBeau SO, Ferris RL, Yip L, Seethala RR, Tublin ME, Stang MT, Coyne C, Johnson JT, Stewart AF, Nikiforova MN (2011) Impact of mutational testing on the diagnosis and management of patients with cytologically indeterminate thyroid nodules: a prospective analysis of 1056 FNA samples. J Clin Endocrinol Metab 96:3390–3397
7. American Thyroid Association (ATA) Guidelines Taskforce on Thyroid Nodules and differentiated Thyroid Cancer, Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Pacini F, Schlumberger M, Sherman SI, Steward DL, Tuttle RM (2009) Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 19:1167–1214
8. Gharib H, Papini E, Paschke R, Duick DS, Valcavi R, Hegedüs L, Vitti P (2010) AACE/AME/ETA Task Force on Thyroid Nodules. American Association of Clinical Endocrinologists, Associazione Medici Endocrinologi, and European Thyroid Association Medical guidelines for clinical practice for the diagnosis and management of thyroid nodules: executive summary of recommendations. Endocr Pract 16:468–475
9. Schlumberger MJ, Filetti S, Hay ID (2011) Nontoxic diffuse and nodular goiter and thyroid neoplasia. In: Kronenberg HM, Melmed S, Polonsky KS, Larsen PR. Williams textbook of endocrinology. 11nd ed. Saunders Elsevier, pp 412–417.
10. Frates MC, Benson CB, Charboneau JW, Cibas ES, Clark OH, Coleman BG, Cronan JJ, Doubilet PM, Evans DB, Goellner JR, Hay ID, Hertzberg BS, Intenzo CM, Jeffrey RB, Langer JE, Larsen PR, Mandel SJ, Middleton WD, Reading CC, Sherman SI, Tessler FN (2006) Management of thyroid nodules detected at US: Society of Radiologists in Ultrasound consensus conference statement. Ultrasound Q 22:231–238
11. Bongiovanni M, Krane JF, Cibas ES, Faquin WC (2012) The atypical thyroid fine-needle aspiration: past, present, and future. Cancer Cytopathol 120:73–86
12. Baloch ZW, Cibas ES, Clark DP, Layfield LJ, Ljung BM, Pitman MB, Abati A (2008) The National Cancer Institute Thyroid fine needle aspiration state of the science conference: a summation. Cytojournal 5(6):1–17
13. Jo VY, Stelow EB, Dustin SM, Hanley KZ (2010) Malignancy risk for fine-needle aspiration of thyroid lesions according to the Bethesda System for Reporting Thyroid Cytopathology. Am J Clin Pathol 134: 450–456
14. Cibas ES, Ali SZ (2009) The Bethesda System for Reporting Thyroid Cytopathology. Thyroid 19:1159–1165
15. Faquin WC (2009) Diagnosis and reporting of follicular-patterned thyroid lesions by fine needle aspiration. Head Neck Pathol 3:82–85
16. Broome JT, Solorzano CC (2011) The impact of atypia/follicular lesion of undetermined significance on the rate of malignancy in thyroid fine-needle aspiration: evaluation of the Bethesda System for Reporting Thyroid Cytopathology. Surgery 150:1234–1241
17. Jadhav S, Lila A, Bandgar T, Shah N (2012) Promise and pitfalls of molecular markers of thyroid nodules. Indian J Endocrinol Metab 16: 162–166
18. Teixeira GV, Chikota H, Teixeira T, Manfro G, Pai SI, Tufano RP (2012) Incidence of malignancy in thyroid nodules determined to be follicular lesions of undetermined significance on fine-needle aspiration. World J Surg 36:69–74
19. Filie AC, Asa SL, Geisinger KR, Logani S, Merino M, Nikiforov YE, Clark DP (2008) Utilization of ancillary studies in thyroid fine needle aspirates: a synopsis of the National Cancer Institute Thyroid Fine Needle Aspiration State of the Science Conference. Diagn Cytopathol 36:438–441
20. Fadda G, Rossi ED, Raffaelli M, Pontecorvi A, Sioletic S, Morassi F, Lombardi CP, Zannoni GF, Rindi G (2011) Follicular thyroid neoplasms can be classified as low- and high-risk according to HBME-1 and Galectin-3 expression on liquid-based fine-needle cytology. Eur J Endocrinol 165:447–453
21. Cochand-Priollet B, Dahan H, Laloi-Michelin M, Polivka M, Saada M, Herman P, Guillausseau PJ, Hamzi L, Poté N, Sarfati E, Wassef M, Combe H, Raulic-Raimond D, Chedin P, Medeau V, Casanova D, Kania R (2011) Immunocytochemistry with cytokeratin 19 and anti-human mesothelial cell antibody (HBME1) increases the diagnostic accuracy of thyroid fine-needle aspirations: preliminary report of 150 liquid-based fine-needle aspirations with histological control. Thyroid 21:1067–1073
22. Luu MH, Fischer AH, Stockl TJ, Pisharodi L, Owens CL (2011) Atypical follicular cells with equivocal features of papillary thyroid carcinoma is not a low-risk cytologic diagnosis. Acta Cytol 55(6): 526–530
23. Renshaw AA (2010) Should "atypical follicular cells" in thyroid fine-needle aspirates be subclassified? Cancer Cytopathol 118:186–189
24. Olson MT, Clark DP, Erozan YS, Ali SZ (2011) Spectrum of risk of malignancy in subcategories of 'atypia of undetermined significance'. Acta Cytol 55:518–525
25. Renshaw AA (2011) Subclassification of atypical cells of undetermined significance in direct smears of fine-needle aspirations of the thyroid: distinct patterns and associated risk of malignancy. Cancer Cytopathol 119:322–327
26. DeLellis RA, Williams ED (2004) Thyroid and parathyroid tumors. In: DeLellis RA, Lloyd R, Heitz PU, Eng C (eds) WHO classification of tumors, pathology and genetics—tumors of endocrine organs. France, IARC Press, Lyon, pp 49–97
27. Dincer N, Balci S, Yazgan A, Guney G, Ersoy R, Cakir B, Guler G (2013) Follow-up of atypia and follicular lesions of undetermined significance in thyroid fine needle aspiration cytology. Cytopathology 24:385–390
28. VanderLaan PA, Marqusee E, Krane JF (2011) Clinical Outcome for atypia of undetermined significance in thyroid fine-needle aspirations: should repeated fna be the preferred initial approach? Am J Clin Pathol 135:770–775
29. Singh RS, Wang HH (2011) Eliminating the "Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance" category from the Bethesda System for Reporting Thyroid Cytopathology. Am J Clin Pathol 136:896–902
30. Rago T, Di Coscio G, Basolo F, Scutari M, Elisci R, Berti P, Miccoli P, Romani R, Faviana P, Pinchera A, Vitti P (2007) Combined clinical, thyroid ultrasound and cytological features help to predict thyroid malignancy in follicular and Hurthle cell thyroid lesions: results from a series of 505 consecutive patients. Clin Endocrinol (Oxf) 66:13–20
31. Bonzanini M, Amadori P, Morelli L, Fasanella S, Pertile R, Mattiuzzi A, Marini G, Niccolini M, Tirone G, Rigamonti M, Dalla Palma P (2011) Subclassification of the "grey zone" of thyroid cytology; a retrospective descriptive study with clinical, cytological, and histological correlation. J Thyroid Res 2011:251680
32. Nayar R, Ivanovic M (2009) The indeterminate thyroid fine-needle aspiration: experience from an academic center using terminology similar to that proposed in the 2007 National Cancer Institute Thyroid Fine Needle Aspiration State of the Science Conference. Cancer 117: 195–202
33. Yassa L, Cibas ES, Benson CB, Frates MC, Doubilet PM, Gawande AA, Moore FD Jr, Kim BW, Nosé V, Marqusee E, Larsen PR, Alexander EK (2007) Long-term assessment of a

multidisciplinary approach to thyroid nodule diagnostic evaluation. Cancer 11:508–516
34. Yang J, Schnadig V, Logrono R, Wasserman PG (2007) Fine-needle aspiration of thyroid nodules: a study of 4703 patients with histologic and clinical correlations. Cancer 111:306–315
35. Theoharis CG, Schofield KM, Hammers L, Udelsman R, Chhieng DC (2009) The Bethesda thyroid fine-needle aspiration classification system: year 1 at an academic institution. Thyroid 19:1215–1223
36. Layfield LJ, Morton MJ, Cramer HM et al (2009) Implications of the proposed thyroid fine-needle aspiration category of 'follicular lesion of undetermined significance': a five year multi-institutional analysis. Diagn Cytopathol 37:710–714
37. Rabaglia JL, Kabbani W, Wallace L et al (2010) Effect of the Bethesda system for reporting thyroid cytopathology on thyroidectomy rates and malignancy risk in cytologically indeterminate lesions. Surgery 148:1267–1272
38. Faquin WC, Baloch ZW (2010) Fine-needle aspiration of follicular patterned lesions of the thyroid: Diagnosis, management, and follow-up according to National Cancer Institute (NCI) recommendations. Diagn Cytopathol 38:731–739
39. Onder S, Firat P, Ates D (2013) The Bethesda system for reporting thyroid cytopathology: an institutional experience of the outcome of indeterminate categories. Cytopathology Sep 2. doi:10.1111/cyt.12091
40. Choi YJ, Baek JH, Ha EJ, Lim HK, Lee JH, Kim JK, Song DE, Shong Y, Hong SJ (2013) Different risk of malignancy and management recommendations in subcategories of thyroid nodules with atypia of undetermined significance (AUS) or follicular lesion of undetermined significance (FLUS): The role of US-guided core-needle biopsy (CNB). Thyroid Aug 15. doi:10.1089/thy.2012.0635
41. Horne MJ, Chhieng DC, Theoharis C, Schofield K, Kowalski D, Prasad ML, Hammers L, Udelsman R, Adeniran AJ (2012) Thyroid follicular lesion of undetermined significance: Evaluation of the risk of malignancy using the two-tier sub-classification. Diagn Cytopathol 40:410–415
42. Ozluk Y, Pehlivan E, Gulluoglu MG, Poyanlı A, Salmaslioglu A, Colak N et al (2011) The use of the Bethesda Terminology in thyroid fine-needle aspiration results in a lower rate of surgery for nonmalignant nodules: A report from a reference center in Turkey. Int J Surg Pathol 19:761–771
43. Tee YY, Lowe AJ, Brand CA, Judson RT (2007) Fine-needle aspiration may miss a third of all malignancy in palpable thyroid nodules: a comprehensive literature review. Ann Surg Nov 246:714–720
44. Baloch ZW, Hendreen S, Gupta PK, LiVolsi VA, Mandel SJ, Weber R, Fraker D (2011) Interinstitutional review of thyroid fine-needle aspirations: impact on clinical management of thyroid nodules. Diagn Cytopathol 25:231–234
45. Tan GH, Gharib H (1997) Thyroid incidentalomas: management approaches to nonpalpable nodules discovered incidentally on thyroid imaging. Ann Intern Med 126:226–231
46. Cheung K, Roman SA, Wang TS, Walker HD, Sosa JA (2008) Calcitonin measurement in the evaluation of thyroid nodules in the United States: a cost-effectiveness and decision analysis. J Clin Endocrinol Metab 93:2173–2180
47. Erdoğan MF, Güllü S, Başkal N, Uysal AR, Kamel N, Erdoğan G (1997) Omeprazole: calcitonin stimulation test for the diagnosis follow-up and family screening in medullary thyroid carcinoma. J Clin Endocrinol Metab 82:897–899
48. Costante G, Meringolo D, Durante C, Bianchi D, Nocera M, Tumino S, Crocetti U, Attard M, Maranghi M, Torlontano M, Filetti S (2007) Predictive value of serum calcitonin levels for preoperative diagnosis of medullary thyroid carcinoma in a cohort of 5817 consecutive patients with thyroid nodules. J Clin Endocrinol Metab 92:450–455
49. Karges W, Dralle H, Raue F, Mann K, Reiners C, Grussendorf M, Hüfner M, Niederle B, Brabant G (2004) German Society for Endocrinology (DGE) - Thyroid Section. Calcitonin measurement to detect medullary thyroid carcinoma in nodular goiter: German evidence-based consensus recommendation. Exp Clin Endocrinol Diabetes 112:52–58
50. Baskin HJ (2000) Ultrasound of thyroid nodules. In: Baskin HJ (ed) Thyroid Ultrasound and Ultrasound-Guided FNA Biopsy. Kluwer Academic Publishers, Boston, MA, pp 71–86
51. Deveci MS, Deveci G, LiVolsi VA, Baloch ZW (2006) Fine-needle aspiration of follicular lesions of the thyroid. Diagnosis and follow-up. Cytojournal 3:1–6
52. Choi YJ, Yun JS, Kim DH (2009) Clinical and ultrasound features of cytology diagnosed follicular neoplasm. Endocrin J 56:383–389
53. Banks ND, Kowalski J, Tsai HL, Somervell H, Tufano R, Dackiw AP, Marohn MR, Clark DP, Umbricht CB, Zeiger MA (2008) A diagnostic predictor model for indeterminate or suspicious thyroid FNA samples. Thyroid 18:933–941

Informative Title

Malignancy rate of thyroid nodules in thyroid cytopathology defined as follicular lesion of undetermined significance and atypia of undetermined significance.